Myotonic dystrophy (DM) is the most prevalent human dystrophy; it results from a *spliceopathy* in which muscleblind-like (MBNL) regulatory proteins are sequestered by expanded CUG triplet repeats. In order to investigate the specific role that MBNL proteins play on the functional expression of chloride (ClC-1) channels, we studied the chloride currents (ICl) in fibers isolated from FDB muscles of adult knockout (KO) mice lacking MBNL1, MBNL3, or both (MBNL1/3 DKO). ICl were recorded in fibers voltage clamped with 2 microelectrodes, internally equilibrated with 70 mM intracellular chloride, and bathed in TEA-Cl solution. We found that ICl records in fibers from the three knockout strains display kinetic and voltage-dependent properties comparable to those in control fibers (129SV mice). However, the maximal peak ICl (peak-ICl_{max}), and the maximal conductance calculated from them (gCl_{max}), varied markedly among strains. Both peak-ICl_{max} and gCl_{max} are significantly smaller (~34%, $p<005$) in fibers of adult MBNL1 KO mice, than in those of the controls. The persistently impaired functional expression of ClC-1 channels contrasts with the transient chloride channelopathy of the HSA^{LR} model of DM. Furthermore, while ICl records in fibers of MBNL3 KO mice are identical to those from their control counterparts, peak-ICl_{max} in fibers of MBNL1/3 DKO mice show more severe reductions (~50%, $p<005$) than those of MBNL1 KO. These interesting results suggest novel synergistic regulatory interactions between MBNL proteins which ultimately affect the functional expression of ClC-1 channels. This work was supported by NIH grants AR047664, AR041802, and AR054816. Precursors of MBNL1 mice were kindly provided by Dr. M. Swanson, University of Florida.

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To the Molecular Mechanism of Mechanoelectrical Transduction in Cell

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Admittedly, mechanical deformation governs intracellular potential by means of specific stretch-activated channels in cell membrane. At the same time, the key mechanism of the mechanical stimulus (stretch) transduction in electrophysiological response is still not clear. Numerous studies by different authors have convincingly demonstrated the cytoskeleton sub-membrane structures (cortex) critical need for the mechanoelectrical transduction providing. From the physicochemical point of view, the cytoskeleton as a whole, and the cortex in particular resembles a polyelectrolyte hydrogel, i.e., a 3D biopolymer network with the electric charges localized on the macromolecular filaments, and with free counterions dispersed in the liquid phase inside the network. Presented investigation addresses the possible mechanism of stretch on cell electrochemical potential change, based on the physicochemical properties of cytoskeletal network. Synthetic polyelectrolyte gels were used as an experimental model of the cytoskeleton. We have found that axial deformation of polyelectrolyte gel shifts gel potential to depolarization. The decrease of potential with gel is the result of diminishing of counterion concentration inside the gel. The underlying mechanism of it is likely the universal process of counterion adsorption on charged polymer filaments due to the decrease of the distance between polymer filaments owing to gel elongation. Thus, the physicochemical properties of the gel network may affect the balance of ions between the cortex and liquid phase of the cell. Independently of the activity of stretch-activated channels, stretch of the cortex network is able to diminish the absolute value of cell potential. On the other hand, we may suppose also that such depolarization is the main factor that determines stretch-activated channels activity.

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Canonical WNT Pathway Enriches Cardiac Pacemaker Cell Population During Embryonic Stem Cell Differentiation

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Background: Embryonic stem cells (ESCs) can be guided to differentiate into cardiomyocytes by blocking canonical Wnt pathway (e.g., with Dkk-1) during cardiac specification stage. We hypothesized that canonical Wnt signaling may be an important negotiator during cardiac progenitors' commitment towards pacemaker or atrial/ventricular lineages.
Methods: Mouse ESCs were treated with activin-A/BMP-4 for 40 hours in a defined medium to initiate cardiac differentiation. Flk-1+/PdgfR-α+ cardiac progenitors are FACS-purified and seeded as monolayers with Dkk-1 (day-0).
Results: At day-4, ~65% of cells are positive for cTnT, a pan-cardiomyocyte marker. Some cTnT-positive cells express one or more of pacemaker-lineage markers, Shox2/Tbx18/Tbx3/Hcn4. Spontaneously-beating areas are observed starting day-2, and some single cells exhibit spontaneous, rhythmic action potentials with hallmark pacemaker electrophysiology such as phase-4 depolarization and depolarized maximal diastolic potential. Still, the monolayers beat in syncytium, resembling the passive contractions of atrial/ventricular myocardium. Removal of Dkk-1 significantly increases pacemaker gene transcript levels, Tbx18 and Shox2 by 5-fold ($p<0.05$, n=4), Hcn1 and Hcn4 by 2-fold ($p<0.05$, n=4) compared to the cells cultured with Dkk-1. Conversely, ventricular/atrial lineage markers, Nkx2.5 and Scn5a were suppressed by 4- and 8-fold, respectively, compared to control ($p<0.05$, n=4). In contrast to the syncytial contractions of the monolayers cultured with Dkk-1, intact canonical Wnt signaling (no Dkk-1) induces formation of discrete, node-like structures which beat autonomously. The beating rates of cells cultured without Dkk-1 are ~3x faster than that of cells cultured with Dkk-1 (161.5 ± 11.5 vs. 48.0 ± 2.9 bpm, $p<0.01$, n=4) at week-2. Single spontaneously-beating cells isolated from no-Dkk-1 group are frequently spindle-shaped replicating the morphology of genuine sinoatrial node pacemaker cells.
Conclusions: Endogenous, canonical Wnt pathway promotes differentiation of mouse cardiac progenitor cells into pacemaker cells rather than to normally-quiescent cardiomyocytes.

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Parametric Sensitivity Analysis of the Most Recent Computational Models of Rabbit Cardiac Pacemaking

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The cellular basis of cardiac pacemaking activity, and specifically the quantitative contributions of particular mechanisms, is still debated. Reliable computational models of sinoatrial node (SAN) cells may provide mechanistic insights, but competing models are built from different data sets and with different underlying assumptions. To understand quantitative differences between alternative models, we performed thorough parameter sensitivity analyses of the SAN models of Maltsev & Lakatta (2009) and Severi et al (2012). Model parameters were randomized to generate a population of cell models with different properties, simulations performed with each set of random parameters generated 14 quantitative outputs that characterized cellular activity, and regression methods were used to analyze the population behavior.
Our analysis pointed out that the two models, exhibit clearly different (sometime even opposite) sensitivity to several parameters. As relevant examples: (1) Na+-K+ pump activity, rapid delayed rectifier current (IKr) activation and SR Ca^{2+} pump activity had a greater effect on cycle length (CL) in the Maltsev model; (2) conversely, parameters describing the funny current (If) had a greater effect on CL in the Severi model; (3) changes in IKr conductance (GKr) had opposite effects on action potential (AP) amplitude in the two models.
Within the population, a greater percentage of model cells failed to exhibit APs in the Maltsev model (27%) compared with the Severi model (7%), implying greater robustness in the latter. Confirming this initial impression, bifurcation analyses indicated that smaller changes in GKr or Na^{+}-K^{+} pump activity led to failed APs in the Maltsev model.
Overall, the results suggest experimental tests that can distinguish between models and alternative hypotheses, and the analysis offers strategies for developing anti-arrhythmic pharmaceuticals by predicting their effect on the pacemaking activity.

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Mathematical Modelling of the Autonomous Activity of Cultured Neonatal Rat Ventricular Myocytes

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Problematic. The biological pacemaker is a new therapeutic approach that could lead to optimized treatment of bradycardia. A possibility is the development of a thin sheet of cardiomyocytes, cultured to obtain a target activation rate. Fundamental research, often conducted with neonatal rat ventricular myocytes (NRVMs), partially revealed two basic coupled mechanisms of automaticity termed Voltage Clock (synergy of membrane currents) and Calcium Clock (internal oscillations of calcium concentration). To date, no ionic model is able to reproduce in silico the autonomous activity found in cultured NRVMs. The present project aims to fill this gap.
Methods. A non-automatic NRVM ionic model (Korhonen-Tavi, 2009) is modified according to documented Voltage and Calcium Clocks mathematical formulations. The myocytes are cultured for 48 hours at low density, allowing cells to remain single on dishes. Autonomous action potentials (APs) are measured with patch clamp method, and calcium transients (CTs) with Fluo-4 AM